Vol. IV. *December, 1902.* *No. 1.*

BIOLOGICAL BULLETIN.

NOTES ON THE REGENERATION OF GONIONEMA.

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The experiments of which this paper presents a résumé were conducted at the Marine Biological Laboratory during the summer of 1901. The purpose in view was such a review of previous work done by C. W. Hargitt '97,[1] '99,[2] and by Morgan '99,[3] as would confirm or correct, or possibly extend, the conclusions then announced.

The work was suggested by and the experiments carried on under the direction of C. W. Hargitt, whose advice and oversight has been largely instrumental in making possible the preparation of this paper.

By way of introduction it may be stated that three general series of experiments were projected:

I. Experiments upon the regeneration of the marginal organs of the medusæ.

II. Experiments on the regeneration of the manubrium.

III. Experiments upon the regeneration of the radial canals.

Under each of these series several sets of experiments were conducted, and under each set a sufficient number of specimens operated upon to insure reasonable precautions against failure or unwarranted conclusions from limited observations. In other words the repeated experiments operated as controls or checks against exceptional results in either direction.

In general the methods followed were similar to those of previous experiments. The medusæ were operated upon very soon after being brought into the laboratory, being cut into desired forms by means of clean scissors as the animals were

[1] *Zoölogical Bulletin*, Vol. I., p. 27.

[2] BIOLOGICAL BULLETIN, Vol. I., p. 35.

[3] *The American Naturalist*, Vol. XXXIII., p. 939.

held gently, by means of delicate forceps, in dishes of perfectly fresh sea water. After the operation the specimens were immediately transferred to clean sea water, which was afterward changed at least daily and sometimes oftener. The specimens were fed from time to time with bits of freshly killed shrimp or small fish. The specimens usually seemed to thrive quite well considering the artificial conditions under which they were placed and the mutilation to which the operations had subjected them. Occasionally, as will be noted later, whole sets under a given experiment would seem to decline or go bad, a fact doubtless due to accidental contamination of the water in which they were kept.

I. Experiments upon the Regeneration of the Marginal Organs of the Medusæ.

Hargitt '97, and Morgan 99, found that when the entire margin was removed new tentacles regenerated though in their investigations they remained rudimentary and bud-like. I repeated experiments of a similar kind to see in how far my results would confirm theirs. Five sets of experiments were tried.

In all cases the cut edges contracted more or less. In the first set the contraction continued till the edges had nearly met and the bell was spherical and had only a very small opening into the subumbrella. Five days after the operation small refractile bulbs were found at the lower distal pole of the sphere, in several specimens. The next day tentacles had begun to grow from these bulbs. On the ninth day two to four tentacles were found on those in which the bulbs had earlier formed. These tentacles were of considerable length, had the rings of nematocysts and the suctorial pads found in the mature tentacles (Fig. 1). As the opening was so small as to prevent the taking of food, the specimens died before further developments could take place. Particular care was taken to remove the entire margin with all the tentacles and bulbs at the time of operation, so that these regenerated tentacles were undoubtedly of new growth.

In the second set the cut edges contracted, though not into a sphere, and a large opening into the subumbrella was left. No velum formed in any of the specimens. In two days several bulbs had formed on some of the margins. In four days marginal

canals and several bulbs had formed in other specimens. Quite a large number of bulbs were present; in some cases thirty were counted, while in others as many as forty were found. These bulbs are probably an aggregation of new tissue and do not represent the bulbs usually found at the bases of mature tentacles, as these latter normally form after the tentacles have been regenerated. In Fig. 2 the marginal canal and bulbs are shown. From several of the bulbs elongations resembling young tentacles had formed. Specimens were kept two weeks after the bulbs were first found and though the bulbs increased from two or three to twenty or forty the tentacles did not develop beyond what is shown in Fig. 2.

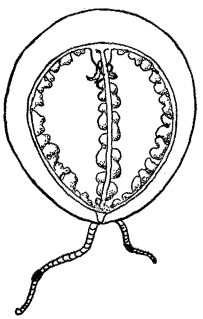

FIG. 1.

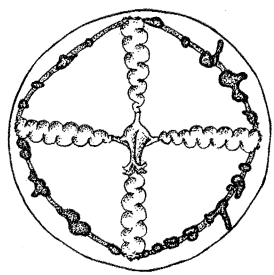

FIG. 2.

In the third set the margins contracted somewhat, but on the second day all evaginated and in some cases the bell began to degenerate. One of the specimens turned right side out and twelve hours later evaginated again and then gradually disintegrated. All the specimens died before any regeneration took place. In the fourth set all died within a week without having regenerated. One specimen flattened out and attached itself to the bottom of the dish by the manubrium. Several of them evaginated and in all the bell slowly disintegrated and the medusæ soon died.

The fact that in these two sets nearly all evaginated and all died within a week, and that this happened only in this experiment, needs some further explanation. The same care and methods were used in this experiment as in all others, with the exception that in the third set the water was taken from the tap

in the laboratory, while in most of the others it was taken directly from the open harbor. Since in these two sets the medusæ were placed in smaller dishes, the consequent overcrowding may have been the cause of their death, through lack of aëration.

In order to ascertain whether the regeneration of new tentacles would take place more rapidly if a portion of the margin was left, than when it was entirely removed, three quarters of the margin was excised leaving one quarter of the original number of tentacles still present. The part from which the tentacles had been removed gradually contracted till it occupied only about one quarter or one half of the new margin. Thus two or three canals were crowded into less than half of the margin and two of them sometimes fused as shown in Fig. 3. New marginal canals were fully formed in about a week, and the circulation of the fluid in them could be observed. In ten or twelve days small bulbs were found on the margins of some, and from these bulbs

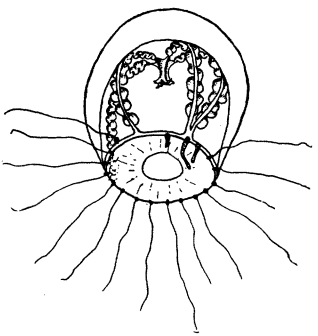

FIG. 3.

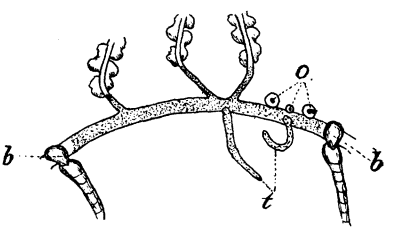

FIG. 4.

tentacles formed, while at about the same time new otocysts developed. As suggested under the second set these bulbs are probably aggregations of new tissue, and are perhaps comparable to the tentacle "anlagen" in regenerating hydroids. Fig. 4 shows the portion of the margin from which the tentacles were excised, *o* represents otocysts and *t* the new tentacles. It will be seen that the pads or bulbs *b* found at the bases of the old tentacles are lacking in the new tentacles at this stage, and form only after the new tentacles have attained considerable growth. This fact has been previously observed and noted by Hargitt[1] 1901. The tentacles

[1] BIOLOGICAL BULLETIN, Vol. II., p. 244.

grew slowly and in two or three weeks some specimens had developed only two to four tentacles, though several small bulbs were present. In other specimens more tentacles formed, Fig. 5

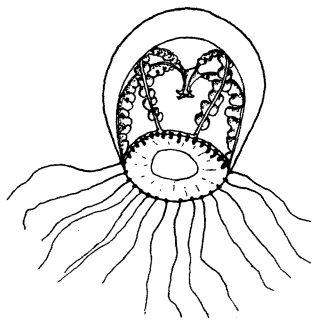

FIG. 5.

showing seventeen bulbs and small tentacles on the regenerated margin.

II. EXPERIMENTS ON THE REGENERATION OF THE MANUBRIUM.

In some cases the manubrium was removed by a circular cut passing entirely through the jelly of the bell. The size of the opening left varied in the different specimens but was not very large in any individual. In other cases the upper half of the bell was removed, thus leaving a very large opening. The rate of healing did not seem to be at all dependent upon the size of the opening, for, as noted below, some of those in which the upper half of the bell was removed took less time to heal than some in which only the manubrium was cut out. In all cases the cut edges gradually contracted till they were touching. As soon as they met they began to fuse and in a very short time the wound was entirely healed. The rate of contraction and healing was not the same in all the specimens.

In the first set the contraction was slow, the cut did not completely heal until 60 hours after the operation. In the second set the contraction was quite rapid. Twelve hours after the operation the cut edges had met and twelve hours later had entirely healed in half the specimens, though in the rest of the specimens of this set 60–70 hours were required. In the third set the cut edges had met and fused at the end of 48 hours. In the

fourth set, although the upper half of the bell was removed, the edges contracted quite rapidly and in 36–48 hours had fused. In this last set the typical bell shape of the medusa could not be assumed because of the large amount of the bell removed. In contracting the margin was drawn up and when the edges had fused the medusa was flattened and the bell very shallow. Sometimes the center of the bell was depressed (Fig. 6), though this disappeared later. Occasionally the bell was twisted somewhat, the margin turned under and a tentacle thrust through the hole in the top of the bell. This of course delayed the healing of the wound. In the four sets of this experiment, after the cut edges had fused no scar or sign of the fusion was present.

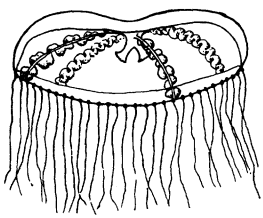

FIG. 6.

By the time the wound in the bell had closed, or very soon after, the free ends of the radial canals had also met and fused. The manubrium developed slowly. In 48–80 hours after the canals had fused a gastric pouch had usually formed. This is simply a large pouch into which the canals lead, and at first has no opening to the exterior. Fig. 7, *a*, shows the gastric pouch before the development of the manubrium. From these pouches the manubria develop. In some cases they had completely formed in four days after the operation. In other cases a week or ten days was necessary for their complete development. Quite common was the formation of double or bifurcated manubria. In the four sets of this experiment 21 per cent. developed double manubria. The formation of the double manubrium was followed out in detail in one specimen. Fig. 7, *a*, shows the large gastric pouch as it first appeared, with no mouth opening. A small diverticulum from this pouch was at first taken for the beginning of a new radial canal, but it developed into another pouch as shown in Fig. 7, *b*. From each of these pouches a

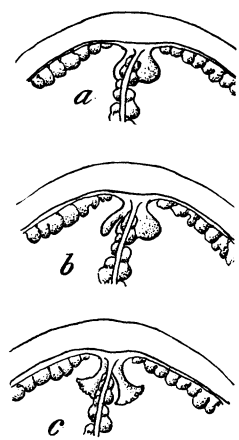

FIG. 7.

manubrium developed as shown in Fig. 7, *c*. All stages were found, from those in which each manubrium was distinct and had a separate pouch, through those in which two manubria were attached to a single pouch, and finally to those in which a single manubrium was branched or bifurcated at its distal end.

The fact that such a considerable per cent. developed double manubria assumes new interest when it is noted that this is not simply the result of artificial mutilation and regeneration. Several specimens of *Gonionema* have been taken from the eel pond near the laboratory, in which double or bifurcated manubria were present. Furthermore, this is not limited to *Gonionema*. During the summer of 1902 a specimen of *Oceania languida* was taken by C. W. Hargitt, in the "tow" in Vineyard Sound, in which two manubria were present. In this specimen three radial canals met normally in the center of the subumbrella. The fourth canal was not complete, extending from the marginal canal only about half way to the center. At the union of the three canals a large four-lobed manubrium was present. At the inner end of the fourth short canal was a small manubrium. This small manubrium was only three-lobed and had three oral frills, and in this resembled some of the regenerated manubria in *Gonionema* which had only two or three lobes. This small manubrium in *Oceania* may perhaps be interpreted as a case of adaptive regeneration. The short canal not having any direct connection with the center of the chymiferous system may have been nearly, if not entirely, deprived of the circulation of the chymiferous fluid, and thus threatened with atrophy ; and the new manubrium may have been formed to remedy this condition. This would be more important inasmuch as the gonad on this canal was as perfectly developed as those on the other canals. In both the cases just mentioned both of the manubria were active and functional.

In the second set the wound entirely healed as noted above, and then in two specimens the bell evaginated. The next day the evaginated ones died without having developed manubria or gastric pouches. In one specimen, Fig. 8, the canals did not unite as in former cases but in the form of a ring, and two manubria formed on opposite sides of the ring. They did not form at

the same time, one being formed a day or two earlier than the other. The presence of these rings is not uncommon in specimens otherwise normal, as noted by Hargitt, 1901. As has already been noted, in the fourth set the upper half of the bell was removed. When the wound had healed the bell was flattened and elongated. It was therefore impossible for the canals to unite in the usual way. The two canals at the ends of the elongated bell united and between these two pairs of canals a

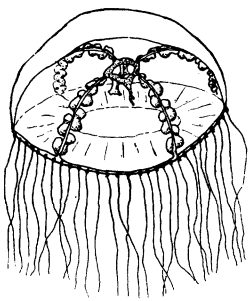

FIG. 8.

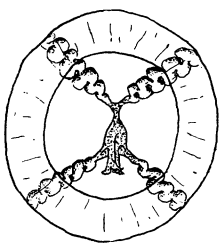

FIG. 9.

straight connecting canal then regenerated, thus completing the chymiferous system. The manubrium then regenerated from these straight connecting canals, but not till the canal was completely formed and functional. If the manubrium formed at the union of the connecting canal with one of the pairs, it was triangular in shape and three-lobed as in Fig. 9. If it formed in the center of the connecting canal it was two- or four-lobed.

III. EXPERIMENTS UPON THE REGENERATION OF THE RADIAL CANALS.

The medusæ were first cut in a vertical interradial plane. Thus each piece had two canals and half the tentacles. The cut sometimes passed through the manubrium and sometimes to one side. Thus some pieces would have a part of the manubrium, others the entire manubrium and in the rest the manubrium would be entirely lacking. The cut edges gradually approached each other and finally met and fused, and the typical medusa-form was thus assumed. Occasionally the manubrium projected between the cut edges and delayed the healing. Usually, how-

ever, the fusion was complete in two to four days. During the process of healing the cut edge of the margin usually drew up somewhat, so that when the fusion was complete there was a scallop or notch in the margin of the bell. The ends of the marginal canal fused and a new radial canal formed along the line of union of the cut edges of the bell. This new canal elongated and the notch in the margin gradually disappeared. Morgan in describing a similar experiment says, "Along the line where the cut edges fused together a scar is present that resembles somewhat a third canal, but the third canal did not develop." While in his experiments this may have been the case, my own show conclusively the development of a third canal. Hargitt, '99, refers to the formation of new canals though he did not demonstrate their functional activity. Along the line of fusion of the cut edges a deposition of pigment is present which might be mistaken for a scar unless carefully examined. At first it is perhaps not a functional canal, but two or three days after the fusion the movement of the chymiferous fluid in the new canal was plainly observed, not only by myself but by others to whom it was pointed out. Furthermore in the closing of the bell, when the oral half had been removed, no sign of a scar or pigmentation was found. Such a scar or pigmented line, therefore, is not simply the sign of the fusion of cut edges, but represent the regeneration of a new organ along what is probably the line of least resistance.

This experiment was varied by cutting out one quadrant with its radial canal, leaving three canals, three quarters of the number of tentacles and the manubrium or a part of the manubrium. The fusion of the cut sides took place in about the same time as in the preceding experiment, though the incidental protrusion of the manubrium through the opening and the consequent delay in healing was more marked. On the fourth day the fusion was complete and a new canal had formed. Fig. 10 shows the new canal at *a*, the scallop in the margin is also shown. The latter gradually disappeared as in the former experiment and the bell margin became quite symmetrical.

The development of gonads on the new canal did not take place while the specimens were under observation. This would undoubtedly occur somewhat slowly if at all. This, however, is

not a constant feature in normal specimens, in many of which gonads are lacking on one or more canals. Occasionally the fusion of the cut edges and the formation of new canals resulted in apparent abnormalities. Fig. 11 shows such a case in which the new canal *a* has anastomosed with one of the old ones. But this again has its counterpart among normal specimens in a state of nature.

The single quadrants which were cut out in the preceding experiment were placed in separate dishes. These contained a single canal, in some cases a part of a manubrium. The contraction of the cut edges took place as usual and in from three to four days the typical medusa-form had been assumed, the edges

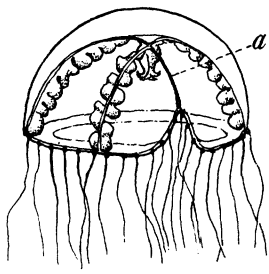

FIG. 10.

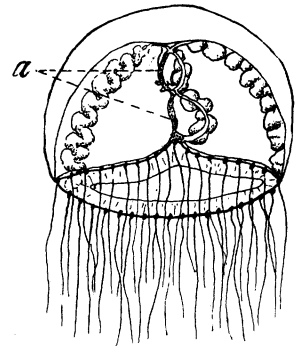

FIG. 11.

of the margin and marginal canal had fused as had also the velum. The apex of the quadrant was flexed toward the margin and when the fusion was complete this portion, and with it the proximal end of the canal, was joined to or very near, the marginal canal. In specimens having a portion of the old manubrium this was also carried downward and fused with the marginal canal and a gastric pouch formed at the point of union, and when the new manubrium was finally regenerated it protruded beyond the edge of the velum in the same horizontal plane. In specimens where no portion of the old manubrium was present usually entirely new ones regenerated but in the same general position as in the former. These new medusæ were of course only about one fourth the size of the original specimen and with only a proportional number of tentacles.

Summary and Review.

Hargitt, '97, showed for the first time that a medusa when divided into two or several pieces had the capacity to recover the original form, and that various organs, as manubria, canals and tentacles, were also regenerated. He also showed that the process was a gradual and orderly one. Further experiments in '99 by the same author showed that at least in case of radial canals and manubria the process was one of actual generation of new tissue and not merely the recasting of parts of old tissue into new forms.

Morgan, '99, experimenting on the same medusæ, confirmed these results to a certain extent, but claimed that new canals were not regenerated. He showed, moreover, that when cut into quadrants or smaller portions while a similar recovery of form was common, the resulting medusa was not typical in that in many cases there were lacking certains organs, as the typical number of canals, gonads, etc. And furthermore in case of smaller pieces where new manubria were regenerated it was not in the normal position. This observer also claimed that where only a portion of the margin was removed the regeneration of tentacles was more prompt and better developed than where the emargination had been complete.

As will have been seen, my own experiments confirm in part those of both these observers, and in part extend their results, by showing conclusively the regeneration and *functional activity* of both radial and marginal canals. My experiments show that when the division of the medusa was in vertical interradial planes that the resulting half medusæ were almost always perfectly symmetrical, while Morgan claimed they were otherwise, in that the manubrium was eccentrically located. This, so far as my experiments go, was only true when the section itself was quite unequal.

I was also able to obtain better results in regeneration of tentacles than either of the authors named, as an inspection of several of the figures will show. Concerning the experiments upon small pieces of medusæ, and regeneration of tentacles of partially emarginated specimens my own confirmed in almost all details those of Morgan.

As to the *processes* involved in these phases of regeneration my experiments confirm in the main those of both Hargitt and Morgan.

Hargitt, '97, says concerning it, "there seems to be an intrinsic potency to recast itself into the morphological equivalent of the original." Morgan says the process "is one of rounding up of the piece in direction of least resistance. The meeting of the edges may sometimes be due to simple accidental meeting of the bent-in portions." My own experiments showed no evidence of a merely accidental or mechanical process, but rather self-directive activity inherent in each portion of the individual. The process was in all cases gradual and orderly and the meeting of the edges too exact and certain to be explained as the result of mere accident.

SYRACUSE UNIVERSITY, THE ZOÖLOGICAL LABORATORY,
September 10, 1902.